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RESEARCH ARTICLE

Serum Free Zinc as A Predictor for Excessive Function of Pancreatic Beta-Cells in Central-Obese Men

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Abstract

BACKGROUND: Central obesity is known as a risk factor for type 2 diabetes mellitus (T2DM). Its development is influenced by many factors such as a progressive failure of pancreatic beta cell function. The beta cells increase their function to secret insulin along T2DM development to compensate before it becomes exhausted. Zinc (Zn) plays a crucial role in beta cell function and insulin secretion. The majority of Zn in serum are bound to protein which is not readily available interact with cells. The free Zn in serum has been suggested as being more representative than total Zn in beta cell function. This research aimed to investigate the correlation between serum free Zn and homeostasis model assessment for beta cell function (HOMA-B) and to predict the pancreatic beta cell function in the development of T2DM.

METHODS: This study was designed as an observational with a cross-sectional approach. The subjects were centrally obese men aged 30-50 years and who had met the inclusion and exclusion criteria from the screening tests. Control subjects were lean men without T2DM. Serum free Zn and serum total Zn were measured by using inductively coupled plasma-mass spectrometry (ICP-MS).

RESULTS: There was positive correlation between serum free Zn and HOMA-B (R=0.361, *p*-value<0.001) but there was no correlation between serum total Zn and HOMA-B (R=-0.062, *p*-value=0.563). This study found that if the concentration of serum free Zn >1.7 ug/dL in central obese men was suggested as an excessive function of pancreatic beta cell and as an early warning before its exhausted.

CONCLUSION: This study suggested that serum free Zn had a correlation with beta cell function and had a predictive ability for beta cell excessive function before its exhausted.

KEYWORDS: type 2 diabetes mellitus, HOMA-B, serum free zinc, central obesity

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Introduction

Central obesity is known as a risk factor for type 2 diabetes mellitus (T2DM) which is preceded by insulin resistance. (1,2) Besides insulin resistance, the other indispensable factor is a failure of the pancreatic beta cell to secrete sufficient insulin to maintain blood glucose concentration. (3) Current findings from Genome-Wide Association Studies (GWAS) have shown that many gene variations increase the T2DM risk that cause pancreatic beta cell dysfunction rather than insulin resistance.(4) Beta cell dysfunction also recognized in pathogenesis and progression of T2DM.(5)

At initial phase of T2DM development, especially in prediabetes, pancreatic beta cells compensate by increasing secretion to meet the demand of insulin needed to control blood glucose concentration. The compensation is most common with the insulin resistance due to obesity which is accompanied by the increase of total insulin secretion.(6) Much of the rise in insulin secretion is a result of an increase

of beta cell mass. In insulin resistance-induced obesity, the increase of beta cell mass probably due to increased of beta cell numbers.(7,8)

The insulin secretion process involves many factors, including the presence of biometals. One of the essential biometals is zinc (Zn) which plays a critical function in the production of secretory granules for insulin secretion.(9) A high concentration of Zn is required in the beta cell for insulin crystallization, maturation, and secretion. Furthermore, Zn is essential for the appropriate insulin synthesis, storage, and structural stability as well. Serum Zn deficiency is common in T2DM and has been suggested as a risk factor for the disease.(10) The exact molecular mechanism of Zn in T2DM remains unclear. Role of Zn in metabolism occurs inside of the cell. Its concentration inside is higher than the outside and this difference in concentration is maintained by Zn homeostasis system. (11) Zn homeostasis is a dynamic system with the pool approximately 0.1% of total Zn concentration in the body and 98% of Zn in the blood is bound to the albumin and not ready to interact with cells including pancreatic beta cells.(12)

The concentration of serum free Zn was expected more representative than serum total Zn in the pancreatic beta cell function and it depicted the natural history of pancreatic beta cell compensation before its exhausted. Currently, there is no data available of serum free Zn concentration profile and its role in pancreatic beta cell function. The objective of this research was to investigate the correlation of serum free Zn concentration with the beta cell function and predict the excessive beta-cell function in central obese-men.

Methods

This was a cross-sectional, observational study. The subjects of this study were 70 central obese men, aged 30-50 years old who were recruited from Jakarta, Bandung, Semarang, and Bogor. They had waist circumference ≥90 cm according to the International Obesity Task Force (IOTF) criteria.(13) HbA1c concentration was used to determine diabetes status according to the American Diabetes Association (ADA) criteria. Diabetes diagnosed when HbA1c concentration ≥6.5%, prediabetes considered when HbA1c concentration was 5.7-6.4%, and non-diabetes stated when HbA1c concentration <5.7%.(14) HbA1c measurement was using high performance liquid chromatography (HPLC) (Variant Turbo, Biorad, USA) which is certified by National Glycohemoglobine Standardization Program (NGSP). The control group consisted of 20 lean men aged 30-50 years old with a waist circumference <90 cm and without T2DM. The number of samples of this study was calculated by using the formula for a quantitative variable.(15)

Subjects who had the following conditions were excluded from the study: acute inflammation marked by hsCRP concentration >10 mg/L, impaired renal function marked by estimated glomerulus filtration rate <60 mL/min/1.73 m^2, taken zinc, iron, and copper supplement in last 1 month, and impaired liver function marked by serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) concentration more than 2 times upper limit or reference value. Laboratory analysis for screening test were done by Prodia Clinical Laboratory. Serum free and total Zn measurement were done and validated by Mass Spectrometry Laboratory in Prodia Clinical Laboratory. Serum free and total Zn were measured by inductively coupled plasma mass spectrometry (ICP-MS). Beta cell function was determined by using the homeostasis model assessment beta cell (HOMA-B) which calculated from fasting blood glucose and insulin concentration.(16) This research protocol was approved by Universitas Padjadjaran Ethics Commision No: 1039/UN6.C.10/PN/2017.

Materials used in this research were human serum collected using metal free vacuum tube, Vivaspin 500 Ultrafilter 10 kDa MWCO (Sartorius, Gottingen, Germany), Nitric Acid 65% supra pure grade (Merck, Darmstadt, Germany), ultra pure water 18.2 Mega Ohm, Zinc standard (Merck), Indium Standard (Merck), Centrifuge (Thermo Scientific, Massachusetts, USA), and ICP-MS (Agilent Technologies, California, USA). Data was processed by using SPSS version 24 for Windows (SPSS Inc, Chicago, USA). The level of significance was 0.05 for data analysis.

Results

The initial number of subject recruited was 120 subjects including control. Of this number, 70 men central obesity were admitted into the study. Twenty lean men who did not have T2DM served as control subjects. Subject characteristics based on the screening test are shown in Table 1.

There was a significant correlation between free serum Zn and HOMA-B but not with total serum Zn as shown in Table 2.

Table 1. Subjects characteristic based on screening tests.

Variables	Lean (n = 20)	Central-Obese (n = 70)			*p* -value
	Non-DM	Non-DM	Pre-DM	DM	
	100%	52.80%	31.40%	15.70%	
Age (years old)	39 ± 6	39 ± 6	40 ± 6	43 ± 5	0.191
Waist Circumference (cm)	81 ± 7	103 ± 9	106 ± 9	106 ± 9	<0.001*
SGOT (U/L)	20 ± 5	25 ± 8	26 ± 9	26 ± 8	0.045*
SGPT (U/L)	20 ± 10	36 ± 15	40 ± 20	39 ± 23	<0.001*
HbA1c (%)	5.2 ± 0.2	5.3 ± 0.3	5.9 ± 0.2	9.1 ± 2.3	<0.001*
eGFR (mL/min/1.73 m^2)	98 ± 11	101 ± 13	104 ± 10	104 ± 14	0.248
hsCRP (mg/L)	0.8 ± 0.6	3.1 ± 2.1	3.6 ± 2.7	5.0 ± 3	<0.001*
Free Zn (ug/dL)	1.4 ± 0.9	1.9 ± 1.2	2.4 ± 1.5	1.3 ± 1.0	0.040*
Total Zn (ug/dL)	83 ± 14	79 ± 10	80 ± 11	75 ± 12	0.759
HOMA-B (%)	66 ± 31	167 ± 99	194 ± 88	110 ± 88	<0.001*

*Significant in 95% confident interval using Kruskal-Wallis test.

HOMA-B classification depicts the function of the pancreatic beta cell. HOMA-B reference value was 70-150%. HOMA-B values of <70% suggest as beta cell dysfunction if it occurs in the T2DM subject (17) and when the HOMA-B value >150% in central obesity is suggested as beta cell excessive function to secreting insulin. Figure 1 shown the median of serum free Zn concentration in the HOMA-B classification. Serum free Zn concentration in T2DM disease development had a similar pattern with the HOMA-B as shown in Figure 2.

ROC analysis showed that serum free Zn concentration of >1.7 ug/dL in central-obese men suggested there was an excessive function of beta cell due to its compensation to secreted insulin in high concentration (AUC: 0.639, 95% CI: 0.521-0.757, *p*-value: 0.025, sensitivity: 64%, and specificity: 68%).

Discussion

The role of Zn in pathogenesis and disease development of T2DM through inflammation mechanism has been established.(8) Of the 70 centrally obese men recruited for this study, 52.8% were not suffering from T2DM, 31.4% were prediabetics, and 15.7% were T2DM. In centrally obese subjects with who were non-diabetic and pre-diabetic, the concentration of serum free Zn was higher than in lean subjects as shown in Table 1. There is no difference for serum total Zn concentration between those groups. T2DM pathogenesis is also caused by chronic inflammation in the adipose tissue, liver, muscle, and pancreas as well. Chronic inflammation is the process when increasing amounts of proinflammatory cytokine reaches the pancreas. (18) This theory was in line with the result of this research that showed increased high-sensitivity C-reactive protein (hsCRP) in central obesity than in lean (3.5±2.4 *vs.* 0.8±0.6 mg/L, *p*-value<0.0001 in 95% confidence interval). In inflammation, Zn influx into cells is higher than in normal condition, and the ready state of Zn before its entered the cell is the free form.(19) Moreover, the binding capacity of a carrier protein to Zn decreased due to inflammation and suggested as a natural response of the cell.(12)

Table 2. Correlation of serum Zn free and Zn total with HOMA-B.

Variables	HOMA-B	
	Correlation Coefficient	*p* -value
Serum Free Zn	0.361	< 0.001*
Serum Total Zn	-0.062	0.563

*Significant in 95% confident interval using Spearman's correlation.

Besides inflammation, T2DM progression is also marked by the increase of pancreatic beta cell function as a compensatory response for the high demand of insulin. Beta cell function in this study was measured by the HOMA-B calculation. Zn also plays a critical role in the activity of beta cell for insulin secretion through its homeostasis.(9) This research showed that there was a positive correlation between serum Zn free and HOMA-B but not with serum total Zn as shown in Table 2. It suggested that the demand of free Zn increased due to beta-cell activity and probably with beta cell mass as well as shown in Figure 1. Figure 1 showed that there was a pattern in every level of HOMA-B. In low HOMA-B (<70%), serum free Zn concentration was lower than in normal HOMA-B (70%-150%) and also high HOMA-B (>150%). High Zn free concentration in excessive function of the beta cell also linked with the theory that stated that Zn deficiency is a risk factor for T2DM progression.(10) Serum Zn deficiency is thought to occur because of the from high demand of Zn from cells including the pancreatic beta cells.

There was a similar pattern of serum free Zn and HOMA-B in the development of T2DM as shown in Figure 2. There was an increase of free Zn which reached the maximum at the prediabetes and decreased in the T2DM condition. In T2DM, the beta cell is exhausted and loses its mass, and as a final result, insulin secretion decreased.(20) Based on that profile, serum free Zn concentration had a high potential to predict the excessive function of pancreatic beta cell in central-obesity. Beta cell excessive function occurred in prediabetes condition, and if that signal is ignored, T2DM progression will cause the beta cell dysfunction. It is crucial to monitor the concentration of serum free Zn in central for the better management of

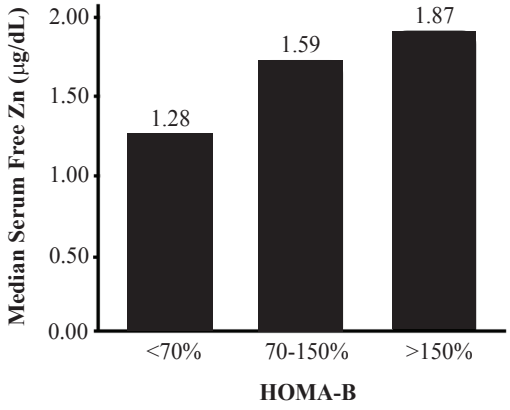

Figure 1. Serum free Zn concentration in HOMA-B classification.

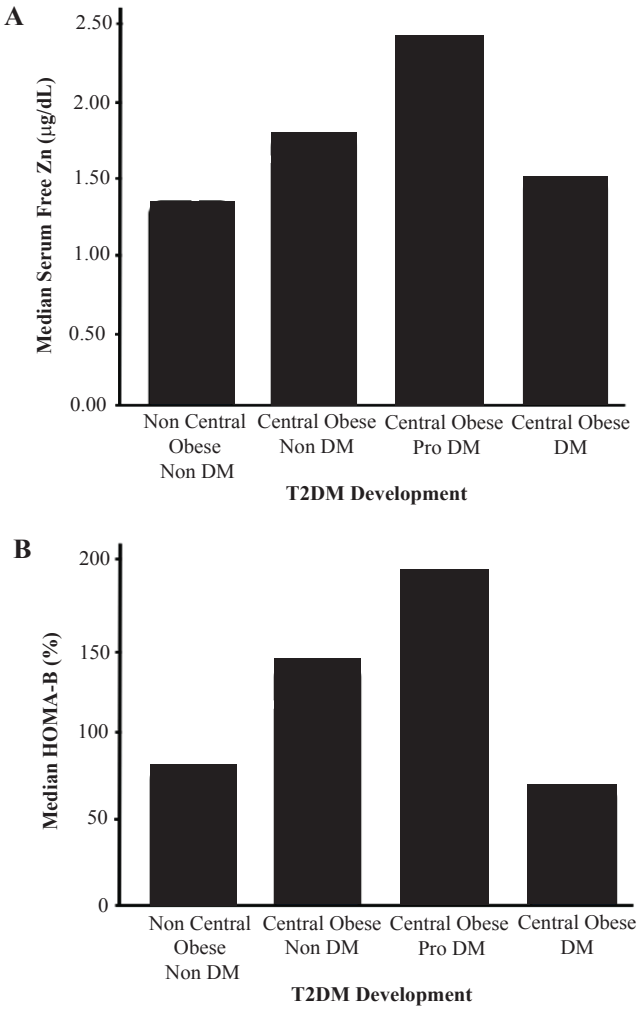

Figure 2. Concentration of serum free Zn and HOMA-B in the development of T2DM. A: median of serum free Zn concentration; B: median of HOMA-B.

T2DM development. Serum free Zn concentration is also considered as a more powerful marker to predict the beta cell function and T2DM development than serum total Zn that is routinely measured.

Conclusion

This study suggested that serum free Zn had a correlation with beta cell function and had a predictive ability for beta cell excessive function before its exhausted.

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